IGF system targeted therapy

Liefers-Visser, J. A. L.; Meijering, R. A. M.; Reyners, A. K. L.; van der Zee, A. G. J.; de Jong, S.

Published in:
CANCER TREATMENT REVIEWS

DOI:
10.1016/j.ctrv.2017.08.012

Publication date:
2017

Cancer Treatment Reviews 60 (2017) 90–99

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Anti-Tumour Treatment

IGF system targeted therapy: Therapeutic opportunities for ovarian cancer

J.A.L. Liefers-Visser [a], R.A.M. Meijering [a], A.K.L. Reyners [a], A.G.J. van der Zee [b], S. de Jong [a,*]

[a] Department of Medical Oncology, Cancer Research Center Groningen, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[b] Department of Gynecologic Oncology, Cancer Research Center Groningen, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form 28 August 2017
Accepted 30 August 2017

Keywords:
IGF system
Ovarian cancer
IGF-1R
Insulin receptor
Targeted therapy
System rewiring

ABSTRACT

The insulin-like growth factor (IGF) system comprises multiple growth factor receptors, including insulin-like growth factor 1 receptor (IGF-1R), insulin receptor (IR) -A and -B. These receptors are activated upon binding to their respective growth factor ligands, IGF-I, IGF-II and insulin, and play an important role in development, maintenance, progression, survival and chemotherapeutic response of ovarian cancer. In many pre-clinical studies anti-IGF-1R/IR targeted strategies proved effective in reducing growth of ovarian cancer models. In addition, anti-IGF-1R targeted strategies potentiated the efficacy of platinum based chemotherapy. Despite the vast amount of encouraging and promising pre-clinical data, anti-IGF-1R/IR targeted strategies lacked efficacy in the clinic. The question is whether targeting the IGF-1R/IR signaling pathway still holds therapeutic potential. In this review we address the complexity of the IGF-1R/IR signaling pathway, including receptor heterodimerization within and outside the IGF system and downstream signaling. Further, we discuss the implications of this complexity on current targeted strategies and indicate therapeutic opportunities for successful targeting of the IGF-1R/IR signaling pathway in ovarian cancer. Multiple-targeted approaches circumventing bidirectional receptor tyrosine kinase (RTK) compensation and prevention of system rewiring are expected to have more therapeutic potential.

Introduction

Ovarian cancer

Ovarian cancer has the highest mortality rate of all gynecological tumors, due to the late onset of symptoms. It represents the fifth leading cause of cancer-related death in American and European women [1]. Most ovarian carcinomas are of epithelial origin (90%) and can be classified into four major histological subtypes, which include serous (70%), endometrioid (10–15%), mucinous (10%) and clear cell (5%). Although these subtypes represent clinically different entities, all patients receive standard treatment consisting of cytoreductive surgery followed by a combination of platinum- and taxane-based chemotherapy. Despite high initial response rates towards chemotherapy, approximately 70% of all patients succumb to recurrent resistant disease within five years after diagnosis. Since the introduction of platinum-based chemotherapy, over three decades ago, survival rates have only marginally improved. For a subgroup of patients vascular endothelial growth factor (VEGF) inhibitors or poly ADP ribose polymerase (PARP) inhibitors are added to standard platinum- and taxane-based treatment regiments [2]. Despite these novel treatment options, platinum-based chemotherapy in combination with taxane-based chemotherapy remains the main pillar in systemic ovarian cancer treatment. Therefore, there is a need for the development of novel and patient tailored therapeutic strategies [3–6].

The IGF system

The insulin-like growth factor (IGF) system is a complex system comprising transmembrane growth factor receptors, growth factor ligands, high affinity IGF binding proteins (IGFBPs), IGFBP proteases and low affinity IGF binding protein related proteins (IGFBP-rP) that regulate both physiological and pathophysiological processes involved in glucose metabolism and cell proliferation [7–11]. Briefly, the IGF signaling pathway comprises the transmembrane receptors, insulin-like growth factor receptor type I (IGF-1R), insulin receptor (IR) -A and -B, the orphan receptor insulin related receptor (IRR) and insulin-like growth factor receptor type II (IGF-2R). Growth factor ligands include IGF-I, IGF-II and

* Corresponding author at: Department of Medical Oncology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
E-mail address: s.de.jong@umcg.nl (S. de Jong).

http://dx.doi.org/10.1016/j.ctrv.2017.08.012
0305-7372/

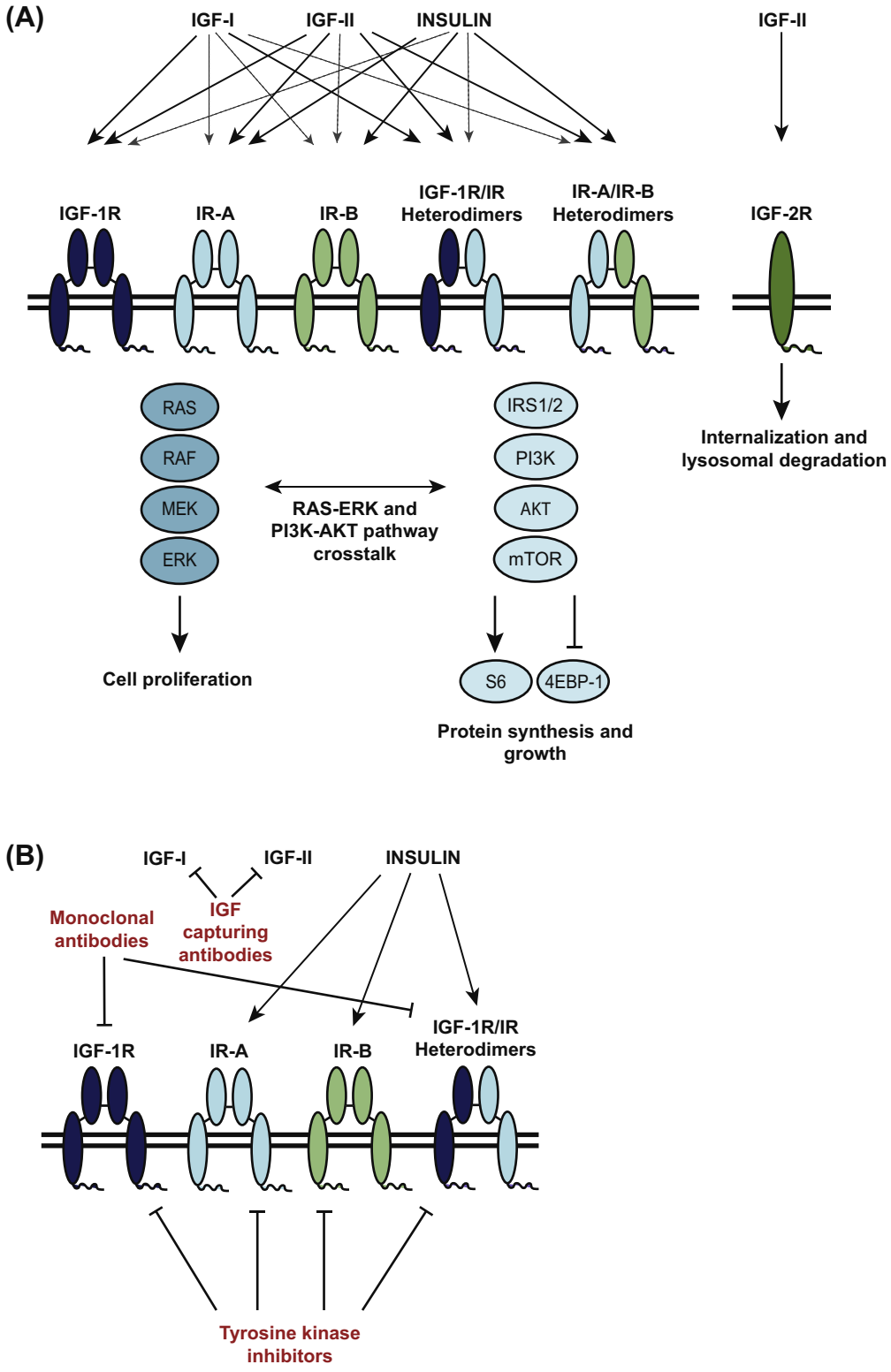

Fig. 1. IGF signaling pathway and targeting strategies. (A) The classical model of IGF signaling involves IGF ligand-receptor interaction followed by downstream signaling transduction via the canonical phosphatidylinositol 3-kinase (PI3K)-AKT and RAS-extracellular signal-regulated kinase (ERK) pathways [12,13]. (B) Targeting the IGF signaling pathway is achieved by multiple strategies including anti-IGF-1R monoclonal antibodies targeting IGF-1R homo- and heterodimers, dual IGF-1R/IR tyrosine kinase inhibitors and IGF-I and -II ligand capturing antibodies abrogating IGF mediated signaling while maintaining insulin signaling.

insulin. Ligand binding to IGF-1R and IR (but not IRR and IGF-2R) transduces downstream signaling via the canonical phosphatidylinositol 3-kinase (PI3K)-AKT and RAS-extracellular signal-regulated kinase (ERK) pathways [7,12,13] (Fig. 1). In addition, cellular IGF-I and IGF-II availability is regulated by 6 high affinity IGF-binding proteins (IGFBP-1-6) and 10 low affinity IGFBP-related proteins (IGFBP-rP 1-10) [8,14].

Hyper activation of the IGF signaling pathway is implicated in the development, maintenance, progression, survival and chemotherapeutic response of many types of cancer, including ovarian cancer [15–18]. This has led to the development of many therapeutic strategies inhibiting or preventing activation of the IGF signaling pathway in cancer cells predominantly via IGF-1R blocking antibodies and tyrosine kinase inhibitors inhibiting both tyrosine kinase domains of IGF-1R and IR [19,20]. In many pre-clinical studies these anti-IGF-1R/IR targeted strategies proved effective in reducing growth of ovarian cancer models. In addition, these anti-IGF-1R targeted strategies potentiated the efficacy of platinum based chemotherapy. However, despite the vast amount of encouraging and promising pre-clinical data, anti-IGF-1R/IR targeted strategies lacked efficacy in the clinic. The question is whether targeting the IGF-1R/IR signaling pathway still holds therapeutic potential.

In this review we address the complexity of the IGF-1R/IR signaling pathway, including receptor heterodimerization within and outside the IGF system and downstream signaling. Further, we discuss the implications of this complexity on current targeted strategies and indicate therapeutic opportunities for successful targeting of the IGF-1R/IR signaling pathway in ovarian cancer.

Complexity of the IGF signaling pathway

Here we will discuss the complexity of the IGF signaling pathway focusing on the various IGF receptors, receptor heterodimerization within the IGF system and the consequences for downstream signaling.

Receptors

IGF-1R, IR and IRR are structurally and functionally related receptors existing as homo- and/or heterodimers. Each receptor dimer is composed of two monomers containing an extracellular α-subunit and transmembrane β-subunit, which are linked together by disulfide bonds in a β-α-α-β rearrangement [21]. Both subunits are synthesized as a single precursor polypeptide and processed to a functional receptor by proteolytic cleavage of the α- and the β-subunit in the trans Golgi network before transportation to the cell surface [22]. High sequence homology between IGF-1R, IR and IRR allows for the formation of heterodimers comprising one α-subunit and one β-subunit of the respective receptor [23–28]. Heterodimer formation occurs by random assembly of the receptor monomers and reflects the molar ratios of the individual receptors. Therefore, less abundant receptors are usually expressed in heterodimer formation [25].

Alternative splicing of exon 11 in *IR* pre-mRNA, results in the expression of two *IR* isoforms, *IR-A*$^{-Ex11}$ and *IR-B*$^{+Ex11}$ respectively. *IGF-1R* does not have an equivalent of *IR* exon 11 and is therefore not alternatively spliced [21].

Although IGF-1R, IR (-A and -B) and IRR are functionally and structurally related receptors, they differ in their ligand binding affinities (Table 1). From high to low order of binding affinity, IGF-1R binds IGF-I, IGF-II and insulin whereas IR binds insulin, IGF-II and IGF-I. The alternatively spliced IR-A has a higher affinity for both IGF-I and IGF-II compared to IR-B. IGF-II binding affinity of IR-A resembles that of IGF-1R, making IR-A a high affinity receptor for IGF-II. Indeed, IR-A transduces IGF-II signals and can compensate for IGF-1R loss [29,30]. Importantly, IR-A is primarily expressed during embryonic development and in cancerous tissues, whereas IR-B is primarily expressed in insulin responsive tissues e.g. liver, muscle and adipose tissue [31]. These observations have led to a paradigm in which IR-A is considered to mediate the mitogenic effects of insulin and IGF-II, whereas IR-B is considered to primarily mediate the metabolic effects of insulin. The implications of IGF-1R, IR-A and IR-B expression in cancer cells will be discussed in more detail in part III.

In contrast to IGF-1R and IR, IRR does not bind IGF-I, IGF-II or insulin and to date no IRR activating ligand has been identified. Instead, IRR is proposed to be an external pH sensor as it is activated at an external cellular pH of 7.9 and higher [32]. Importantly, IRR does form heterodimer receptors with IGF-1R and IR, possibly affecting their function.

In contrast to the aforementioned receptors, IGF-2R (or cation-independent mannose-6-phosphate (M6P)) is a structurally unrelated receptor. IGF-2R encompasses a single transmembrane protein, which lacks intrinsic catalytic activity. IGF-2R has binding affinities for IGF-II and M6P. IGF-2R acts as an IGF-II scavenger receptor, providing the IGF system next to IGFBPs an additional mechanism to regulate cellular IGF-II availability. After binding of IGF-II to IGF-2R, the complex is internalized and degraded. In this regard *MP6/IGF2R* is also considered a tumor suppressor gene. Interestingly, IGF-2R does bind G-protein coupled receptors (GPCRs), possibly providing the IGF system an additional mechanism for IGF signal transduction [33].

Receptor heterodimerization within the IGF system

IGF-1R heterodimers

Receptor heterodimerization within the IGF system impacts ligand binding affinities (Table 1) [26–28]. IGF-1R can form heterodimers with IR-A, IR-B and IRR. Both IGF-1R:IR-A and IGF-1R:IR-B heterodimers bind IGF-I and IGF-II with a high affinity whereas they bind insulin with low affinity. Ligand affinities of these heterodimers are similar to those of IGF-1R homodimers (Table 1) [27,28,34]. Heterodimer formation reflects the molar ratios of the individual receptors. Consequently, in cells with high IGF-1R/IR

Table 1
IC50 (nM) ligand receptor binding affinities.

Receptor type	Insulin	IGF-II	IGF-I	Reference
IR-A	0.2 > 0.9	2.2 > 9.8	9.0 > 41.0	[27–29,108]
IR-B	0.5 > 1.6	10.0 < 25.0	30.0 > 390.0	[27–29,108]
IR-A-IR-B	1.0	10.0	>50.0	[28]
IR-A-IGF-1R	70.0	0.7	0.5	[27]
IR-B-IGF-1R	76.0	0.3	0.3	[27]
IGF-1R	>30.0 < 1000.0	0.5 < 4.4	0.2 < 0.8	[26,27,109]
IGF-2R	–	1.0 > 4.0	–	[110]
IRR	–	–	–	

ratios, IR is primarily expressed as IGF-1R:IR heterodimer, generally favoring IGF-mediated signaling at the expense of insulin-mediated signaling [23].

IR heterodimers

In addition to heterodimerizing with IGF-1R, IR-A and IR-B can be expressed as homo- and heterodimers. Both IR-A and IR-B homo- and heterodimers are characterized by high insulin binding affinities whereas IGF-I binding affinity is low compared to IGF-1R homo- and heterodimers. In contrast to insulin and IGF-I, IGF-II binding affinities differ between IR-A and IR-B homo- and heterodimers. IR-A homodimers and IR-A:IR-B heterodimers are relatively high IGF-II affinity receptors compared to IR-B homodimers (Table 1) [28]. These data suggest that cells expressing high IR-A/IR-B ratios maintain high sensitivity to insulin but are more sensitive to IGF-II compared to IGF-I [28].

IRR heterodimers

As aforementioned, IRR can form heterodimers with IGF-1R and IR [24,23]. From literature it is known that insulin needs two binding sites for high-affinity receptor binding. In contrast, IGF-II requires only one high-affinity binding site [35]. Since IRR does not bind insulin, IGF-I or IGF-II, it is tempting to speculate that IRR heterodimers (IGF-1R:IRR, IR:IRR) are low-affinity insulin binding heterodimers, whereas IGF binding affinity remains unaffected [24].

Downstream signaling

The classical model for downstream signaling upon IGF ligand-receptor interaction involves the transduction of downstream signaling via the canonical PI3K-AKT and ERK pathways in a ligand dependent ON/OFF mechanism [12,13]. The PI3K-AKT and ERK pathways have been extensively reviewed elsewhere [12]. It is now becoming more clear that the classical model of IGF-1R/IR activation and downstream signaling is highly simplified, as it implies that IGF-1R/IR receptor activation initiates a uniform signaling response via the PI3K-AKT and ERK pathways, independent of the receptor composition and the ligand involved. However, distinct downstream responses are identified in various cell line models [36–39].

How this is regulated is not fully understood but multiple factors affect downstream signaling responses, including: (1) ligand-receptor binding affinity, (2) differential binding of downstream protein substrates to the receptor depending on the ligand-receptor combination involved [40], (3) differential receptor internalization and recycling kinetics depending on the ligand-receptor combination involved [41–43], (4) receptor localization in different membrane domains [44].

IGF signaling pathway in ovarian cancer

IGF-1R, IR-A, IR-B, IRR, IGF-I and IGF-II, as well as their regulating IGFBPs, are expressed in ovarian cancer cell lines and ovarian cancer tissues [15,45–48]. Here we will discuss the role of the IGF system and signaling pathway in ovarian cancer in more detail.

IGF receptors

IGF-1R is the predominant expressed receptor in ovarian cancer cells. Therefore IR is expected to be mainly present in IGF-1R:IR heterodimer formation, favoring IGF-mediated signaling over insulin-mediated signaling in these cells. In addition to IGF-1R overexpression, IR-A is preferentially and highly expressed in ovarian cancer cells. As a result of enhanced IR-A expression IR-A/IR-B ratio has changed, which favors IR-A homo- and IR-A:IR-B heterodimer formation [46]. This will augment IGF-II signaling without affecting insulin signaling [28]. High IGF-1R expression has been implicated in the chemotherapeutic response of ovarian cancer, as *IGF-1R* gene expression correlates with cisplatin resistance [49,50].

IRR is expressed in multiple human tissues including the ovary [48,51]. IRR expression was shown to increase from normal ovarian surface epithelium to malignant ovarian surface epithelium, suggesting a role for IRR in ovarian cancer development [48]. Since IRR is expressed in cancer cells and can form heterodimers with both IGF-1R and IR, IRR may have an effect on insulin leaving IGF signaling unaffected.

In contrast to *IGF-1R*, *IGF-2R* gene expression is frequently decreased in ovarian cancer, which is consistent with its possible role as a tumor suppressor [47].

IGF ligands

IGF-I and IGF-II have been implicated in the development, maintenance and chemotherapeutic response of ovarian cancer [52–55]. Both IGF-I and IGF-II expression levels are significantly increased in ovarian cancerous tissues compared to their benign counterparts [54,56]. In addition, ovarian cancer cells excrete IGF-I and IGF-II indicating the presence of autocrine and/or paracrine signaling [16,57]. Further, high *IGF-I* gene expression in ovarian cancer tumors has been implicated in intrinsic resistance to platinum-based chemotherapy [52,58]. In ovarian cancer cell lines, IGF-I treatment induced cisplatin resistance via IGF-1R/PI3K pathway activation. IGF-1R/PI3K inhibition re-sensitized these cells to cisplatin [49]. In addition, *IGF-II* mRNA was upregulated in paclitaxel resistant ovarian cancer cell lines compared to sensitive cell lines. IGF-II knockdown rendered these cell lines sensitive to paclitaxel indicating a role for IGF-II in mediating paclitaxel resistance [55].

IGFBPS

To increase IGF stability and half-life in the circulation, 98% of all IGFs are bound in binary complexes with one of the six IGFBPs or in ternary complexes with either IGFBP-3 (~75%) or IGFBP-5 and the 85 kDa glycoprotein acid-labile subunit (ALS). IGFBPs are primarily produced in the liver and regulate IGF-I and IGF-II tissue distribution and cellular bioavailability. IGFBP binding to extracellular proteases results in the degradation of IGFBPs consequently releasing IGF-I and IGF-II for cellular IGF-1R/IR activation. Many human cancers, including ovarian, cancer express IGFBPs. Possibly, this allows the formation of a locally available pool of IGF-I and IGF-II for IGF-1R/IR activation. Importantly, IGFBPs can both facilitate or attenuate IGF-1R/IR receptor signaling. Enhanced expression of both tumorigenic (IGFBP-2, IGFBP-4 and IGFBP-5 [59,60])

Table 2
IGF signaling pathway targeted strategies.

Targeting strategy	Compound
Anti-IGF-1R monoclonal antibodies	AMG-479 (Ganitumab) IMC-A12 (Cixutumumab) CP-751,871 (Figitumab)
Small molecule inhibitors (TKIs)	MK-0646 (Dalotuzumab) OSI-906 (linsitinib) BMS-754807
IGF-I/II neutralizing monoclonal antibodies	AXL1717 (Picropodophyllin) MEDI-573 BI 836845
Bi-specific IGF-1R/HER3 antibody	MM-141 (Istiratumab)

Table 3
Trials with IGF targeted drugs focusing on ovarian cancer.

	Compound	Phase	Trial
AMG 479 (Ganitumab)	Study of the combination of BYL719 (PI3K inhibition) plus AMG 479 in adult patients with PIK3CA mutated or amplified ovarian carcinoma	Phase Ib/II	NCT01708161 Completed
	Study of adding AMG 479 to first line chemotherapy in patients with optimally debulked epithelial ovarian cancer	Phase II	NCT00718523 Terminated
	Study of AMG 479 as second line therapy in patients with recurrent platinum-sensitive ovarian cancer	Phase II	NCT00719212 Completed
OSI-906 (Linsitinib)	Study evaluating intermittent and continuous OSI-906 and weekly paclitaxel in patients with recurrent epithelial ovarian cancer	Phase I/II	NCT00889382 Completed
MK-0646 (Dalotuzumab)	A Study of Dalotuzumab + MK-2206, Dalotuzumab + MK-0752, and Dalotuzumab + MK-8669 Combination Therapies in Participants With Advanced Cancer (MK-0646-027)	Phase I	NCT01243762 Terminated

and tumor suppressor (IGFBP-3, IGFBP-5 [61–63]) associated IGFBPs has been observed in ovarian cancer.

Targeting the IGF signaling pathway in ovarian cancer

Pre-clinical data

Targeting IGF-1R using antisense strategies, monoclonal antibodies (mAbs) directed at the alpha domain of IGF-1R or small molecules inhibiting the kinase domain of IGF-1R have proven to be effective inhibitors of ovarian cancer cell proliferation in ovarian cancer models (Table 2). Moreover, IGF-1R inhibition potentiated the efficacy of platinum- and taxane-based chemotherapy in these models both *in vitro* and *in vivo* [16,50,53,57,64–67].

IGF-1R knockdown using antisense strategies inhibited IGF-I induced cell proliferation in OVCAR-3 and CaOV-3 ovarian cancer cell lines [16]. In primary ovarian cancer cell lines antisense IGF-1R strategies inhibited cell proliferation and sensitized these cells to cisplatin [67]. IGF-1R inhibition by siRNA interference reduced tumor growth and angiogenesis, and enhanced apoptosis in an OVCAR-3 ovarian cancer xenograft model [66].

IGF-1R monoclonal antibody AMG-479 (Ganitumab) potently inhibited IGF-I, IGF-II and insulin signaling via IGF-1R homo- and IGF-1R/IR heterodimers in multiple ovarian cancer cell lines *in vitro* and *in vivo*. Furthermore, AMG-479 showed synergistic and additive drug interactions with carboplatin or paclitaxel *in* vitro and increased the efficacy of cisplatin in multiple ovarian cancer xenograft models *in vivo* [50]. IGF-1R monoclonal antibody 19D12 inhibited IGF signaling via IGF-1R homo- and IGF-1R/IR heterodimers and inhibited tumor growth in an A2780 ovarian cancer xenograft model [65].

Due to high homology between tyrosine kinase domains of IGF-1R and IR, IGF-1R tyrosine kinase inhibitors (TKIs) inhibit both IGF-1R and IR and thus function as dual IGF-1R/IR inhibitors. IGF-1R/IR inhibition using TKI NVP-AEW451 in IGF-I and IGF-II producing OVCAR-3 and OVCAR-4 cells inhibited cell growth, induced apoptosis and sensitized these cells to cisplatin [57]. Similarly, IGF-1R/IR TKI BMS-754807 inhibited growth of multiple ovarian cancer cell lines *in vitro* [68]. In agreement with the finding that *IGF-I* mRNA was over-expressed in low-grade compared to high-grade serous ovarian cancer tumors, low grade serous ovarian cancer cell lines (HOC-7 and MPSC1) were sensitive to IGF-1R/IR inhibition by the RTK inhibitor OSI-906 (Linsitinib) compared to high grade serous ovarian cancer cell lines (SKOV-3, OVCA420, HeyA8, and 2774) [53].

Clinical data

Multiple clinical studies with IGF-1R mAbs and RTK inhibitors have been performed in ovarian cancer patients (Table 3). Whereas pre-clinical studies using mAbs and RTK inhibitors were promising, most clinical trials using mAbs targeting IGF-1R as a single treatment strategy have shown little clinical benefit. A phase 2 study, using anti-IGF-1R mAb ganitumab (AMG-479) as a second line therapy in patients with recurrent platinum-sensitive ovarian cancer, reported to have modest single agent benefit (NCT00719212) [69]. Sixty-one EOC patients were included in this study. Patient characteristics included serous (68.8%), endometrioid (8.3%), clear cell (6.5%), mucinous (3.3%), mixed (3.3%) and other (9.8%). Ganitumab was well tolerated and did not show any unexpected safety concerns. According to RECIST criteria median progression free survival (PFS) was 2.1 months (95%CI, 2.0–2.8). 2 partial responses (3.4%) and 22 stable diseases (38%) were observed.

Combining anti-IGF-1R mAbs with first line chemotherapy or PI3K-AKT pathway inhibitors has not shown clinical benefit either. A phase 2 study adding ganitumab to first line chemotherapy in 170 patients with optimally debulked EOC has been terminated prematurely (NCT00718523). Patient characteristics included serous (83%), endometrioid (5%), clear cell (2%), mucinous (0.5%), mixed (4.5%) and other (5%). No difference in PFS between control (placebo) and ganitumab arms was observed (NCT00718523). A Phase I/II study evaluating the combination of ganitumab and PI3K inhibitor BYL719 has been completed. To our knowledge no data has been published so far (NCT01708161). A parallel-arm phase I study, using dalotuzumab (MK-0646) a humanized anti-IGF-1R monoclonal antibody, in combination with the AKT inhibitor MK-2206 or the mTOR inhibitor ridaforolimus did not show any objective response according to RECIST criteria. In this study only 12 EOC patients were included. Due to the limited number of patients no preliminary conclusion on efficacy could be established [70]. Antibodies targeting IR in cancer have, to our knowledge, not been developed due to the expected high toxicity.

Largely selective IGF-1R or IR TKIs are not available. One phase I/II study with the dual IGF-1R/IR RTK inhibitor linsitinib (OSI-906) evaluating intermittent and continuous OSI-906 dosing and weekly paclitaxel in patients with recurrent EOC (and other solid tumors), has been completed (NCT00889382) [71]. A total of 58 patients were included in this study. Limited data has been published so far. The combination linsitinib and paclitaxel did not show any unexpected safety concerns. Partial responses were observed in 6 patients (10%) including 3 EOC patients and stable disease in 25 patients (43%) including 10 EOC patients.

Implications of IGF signaling pathway complexity

In retrospect, the complexity of the IGF signaling pathway may have contributed to the failure of IGF targeting strategies in the clinic. Here we will discuss novel insights regarding this complexity and discuss opportunities for successful IGF signaling targeting in the future.

Heterodimer formation and RTK compensatory signaling

In the clinic, single IGF-1R targeting strategies using anti-IGF-1R mAbs have proven to be insufficient. This may be due to compensatory signaling by other RTKs. IR(-A) can compensate for IGF-1R signaling inhibition, thereby inducing resistance to anti-IGF-1R targeted therapies [30]. In addition to the aforementioned IGF-1R heterodimers, IGF-1R has been shown to form direct/heterodimer interactions with RTKs outside the IGF system. Direct interactions with the HER (erbB) family of receptors have been described, more specifically with EGFR (erbB1), HER2 (erbB2) and HER3 (erbB3) (Fig. 2) [72–77]. Moreover, IGF-1R heterotrimerization with HER2 and HER3 has been demonstrated [78]. IGF-1R heterodimer formation with RTKs outside the IGF system may have contributed to the lack of efficacy in the clinic. In addition to these direct RTK interactions, indirect interactions between the IGF-1R, HER and c-Met signaling pathways have been reported [79]. Downstream signaling of all these RTKs converge via the canonical PI3K-AKT and ERK signaling pathways. Therefore, all these RTKs may compensate for loss of downstream signaling upon IGF-1R inhibition. Although dual targeting strategies using IGF-1R/IR RTK inhibitors overcome IGF-1R signaling compensation by IR, it can be equally compensated by other RTKs e.g. EGFR, HER2, HER3 and c-Met. IR is a less promiscuous receptor and primarily interacts with receptors within the IGF system. However, direct/heterodimer interactions with c-Met, have been described in hepatocytes [80]. In addition, indirect interactions between IR and EGFR signaling pathways have been described in hepatocellular and colon cancer cells [81,82]. For example, IR-A signaling conferred resistance to EGFR RTK inhibitor gefitinib in colon cancer cells [81]. These direct and indirect IGF-1R and IR interactions have not been demonstrated in ovarian cancer cells or tissues, so far. However, since ovarian cancers frequently overexpress EGFR, HER2, HER3 and c-Met, it may well be that these interactions play a role in ovarian cancer as well.

Importantly, RTK compensatory signaling is observed to be bidirectional, meaning that not only HER family members can confer resistance to IGF-1R targeted therapies, but IGF-1R can confer resistance to HER family targeted therapies as well. Though this is primarily observed in breast and lung cancer cells [78,83,74,84], similar observations have been made for ovarian cancer cells [84,85]. For example, HER2 was highly activated in ovarian cancer cell lines in response to the dual IGF-1R/IR TKI BMS-536924 [84]. Dual HER family and IGF-1R/IR inhibition by BMS-599626 and BMS-536924, respectively, indeed induced synergistic growth inhibition [84]. Conversely, IGF-1R and HER3 were significantly upregulated in trastuzumab resistant SKOV-3 ovarian cancer cells [85].

Underestimation of the downstream complexity

Recently, it has been demonstrated that IR, IGF-1R and IGF-2R also engage in G-protein coupled receptor (GPCR) signaling [12,13]. Both IR and IGF-1R have been shown to bind G-proteins and β-arrestin-1. In addition, IGF-2R binds GPCRs, possibly providing IGF-2R a method for signal transduction. For IGF-1R, however, all components of a functional GPCR have been attributed. Consequently, IGF-1R is now proposed to function as an RTK/GPCR hybrid (reviewed in:[13]). It is important to note that IR and IGF-1R utilize different G-proteins possibly providing a mechanism for the observed signaling specificity as different G-proteins regulate different downstream effectors [86].

In addition to its role as a RTK/GPCR hybrid, the paradigm of biased signaling has been added to IGF-1R signaling. The paradigm of biased signaling originates from the GPCR signaling field. Central

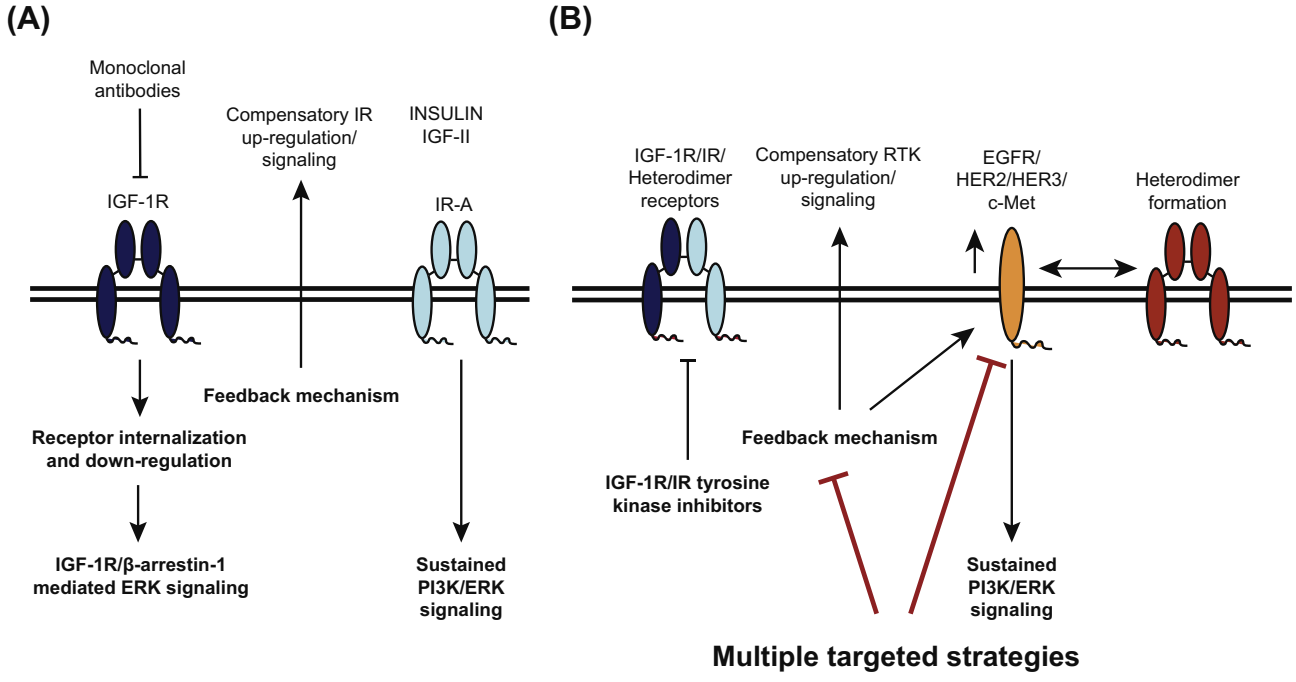

Fig. 2. IGF signaling pathway inhibition and signaling rewiring routes. (A) Anti-IGF-1R monoclonal antibodies inhibit IGF-1R signaling and induce IGF-1R receptor internalization and down-regulation effectively inhibiting IGF-1R function. IGF-1R antibodies in addition to blocking IGF-1R receptor function can function as biased agonist and unintentionally induce receptor internalization activating IGF-1R/β-arrestin-1 mediated ERK signaling. Further IR can compensate for loss of IGF-1R signaling by mediating IGF-II (and IGF-I) signaling via IR-A thus sustaining proliferative signals via the PI3K and ERK signaling pathways. (B) Dual IGF-1R/IR targeting by tyrosine kinase inhibitors prevents compensatory IR signaling. However, upon IGF-1R and IR signaling loss multiple other RTKs (including members of the HER family) can compensate as downstream signaling of all these receptors converge via the canonical phosphatidylinositol 3-kinase (PI3K)-AKT and RAS-extracellular signal-regulated kinase (ERK) pathways. Further, IGF-1R can form heterodimers with EGFR, HER2 and HER3 providing another method of resistance to IGF-1R inhibition. Therefore, multiple targeted therapies are warranted.

to this paradigm is that either the ligand or the receptor is biased towards a specific signaling pathway [13]. For example, besides blocking IGF-1R, IGF-1R mAbs can induce receptor internalization, which unintentionally activates IGF-1R/β-arrestin-1 mediated ERK signaling thus functioning as an IGF-1R/β-arrestin-1 biased agonist (Fig. 2). Furthermore, the newly recognized IGF-1R agonist bacterial peptide LL-37 selectively activates the ERK pathway without affecting the PI3K-AKT pathway, suggesting biased signaling as well. The classical model for RTK signaling is based on ligand-receptor interaction followed by tyrosine kinase activation and linear signal transduction via the PI3K and ERK pathways but does not take into account signaling independent of tyrosine kinase activity. This might be an important factor contributing to the failure of these antibodies in the clinic.

Opportunities for future IGF targeted strategies in (ovarian) cancer

Targeting IR-A

The role of IGF-1R in cancer development, progression and chemotherapeutic response is evident. Meanwhile, IR has been largely ignored as a possible secondary target for years. This is partly due to the fact that IR was considered non-targetable because of its major role in glucose homeostasis and the subsequent expectation of high toxicity. However, clinical trials using dual IGF-1R/IR RTK inhibitors have shown manageable side effects, making dual IGF-1R/IR inhibition feasible. Common side effects included fatigue, nausea and hyperglycemia. Hyperglycemia was reported in only 2% of all patients in a phase 3 study investigating the effects of dual IGF-1R/IR RTK inhibitor Linsitinib (OSI-906) [87].

It has been demonstrated that the IR-A isoform, predominantly expressed in cancer cells, mediates the growth promoting effects of IGF-II in addition to the metabolic effects of insulin [29,88,89]. The mechanisms involved in preferential IR-A expression in cancer cells are largely unknown. In contrast, IR-B expression is frequently reduced in cancer cells [90]. In ovarian cancer cells, preferential IR-A expression either or not accompanied by elevated IGF-1R levels is expected to have a high impact on the formation of receptor heterodimers. In Ewing's sarcoma, IR-A specifically induced resistance to anti-IGF-1R therapy in tumors with a low IGF-1R:IR ratio [91]. Thus, targeting IR-A in addition to IGF-1R would be a preferred strategy. However, specific targeting of either one of the IR isoforms has not been achieved so far.

Multiple splicing factors are involved in *IR* exon 11 splicing and could be potential targets. These include SRSF1, SRSF3 which are known to promote exon 11 inclusion and CELF1 (CUG-BP-1), hnRNPF and nhRNPA1 which inhibit exon 11 inclusion [92]. In addition, upregulation of hnRNPH, hnRNPA2B1, and SF2/ASF splicing factors resulted in enhanced *IR-A* formation [82].

IR-A and IR-B are similarly maturated in the trans-Golgi network by the proprotein convertase furin [22]. However, a recent study demonstrated that IR-B proteolytic cleavage is not solely dependent on furin. When furin-dependent maturation was blocked, both IR-A and IR-B pro-receptors moved to the cell surface but only IR-B was further proteolytic maturated by another proprotein convertase PACE4. These data suggest that furin inhibition may be a method to reduce IR-A maturation and its mitogenic signaling without affecting the metabolic signaling of IR-B.

In summary, IR specific or IR isoform specific targeting is not feasible yet. Targeting IR splicing factors or the proprotein convertase furin might be an indirect way of reducing IR-A expression without effecting IR-B. These results warrant further investigation.

Targeting IGF-I and IGF-II

In addition to their proposed function as an RTK/GPCR hybrid it was stated that IGF-1R and IR are dependence receptors [93]. Meaning that these receptors transduce positive signals in the presence of ligands leading to cell survival, whereas in the absence of ligands receptor-mediated negative signals initiate programmed cell death [93]. These insights advocate the use of IGF-capturing antibodies. Currently two IGF ligand-capturing antibodies are evaluated in phase I clinical trials, MEDI-573 and BI 836845 respectively [94,95]. Phase I dose-escalation and safety studies show MEDI-573 to be well tolerated (NCT01340040) [96]. For BI 836845a phase I trial has been completed but no results have been published so far (NCT01403974). Although IGF-I and IGF-II ligand-capturing antibodies were initially developed to specifically inhibit mitogenic IGF-1R signaling, an additional advantage would be the abrogation of mitogenic IGF-II signaling via IR-A.

Multi-targeted approaches aimed at preventing RTK system rewiring

Both IGF-1R and IR-A are important targets in ovarian cancer. Though, dual IGF-1R/IR RTK inhibitors tackle the problem of IGF-1R compensatory signaling by IR-A, dual IGF-1R/IR inhibition is equally compensated by multiple other RTKs. Therefore, multi-targeted approaches, either aimed at preventing this RTK system rewiring or aimed at targeting multiple RTKs in combination with downstream pathway inhibitors, such as PI3K, AKT and ERK inhibitors, may be more effective. Dual IGF-1R/IR and HER receptor family inhibition can be a strategy to overcome potential compensatory signaling in response to RTK inhibition of either receptor. This was illustrated by effective dual HER family and IGF-1R inhibition by RTK inhibitors and a bi-specific antibody against IGF-1R and HER3 in ovarian cancer cell lines [84,97].

Recent data now advocate the combinatorial use of RTK, PI3K or ERK inhibitors with BET bromodomain inhibitors. These BET bromodomain inhibitors suppress RTK system rewiring by preventing the up-regulation of several compensatory RTKs, including HER family receptors, IGF-1R, IR as well as c-Met [98–100]. In OVCAR-3 ovarian cancer cells PI3K inhibition resulted in compensatory up-regulation of IR and HER3. Combined PI3K and BET bromodomain inhibition reduced cell proliferation in these cells more efficiently than either treatment alone [98]. Ovarian cancer patient derived xenograft (PDX) models characterized by high MYCN or c-MYC expression levels exhibited sensitivity towards BET bromodomain inhibition by JQ1 [101]. Recently, it was shown in ovarian cancer cell lines that BET bromodomain inhibitors may have limited success as single treatment as adaptive kinome reprogramming occurs in response to single BET bromodomain inhibition as well [100]. Therefore, BET bromodomain inhibition also requires combination therapies targeting both kinases and BET bromodomain proteins. Indeed, ovarian cancer cells chronically exposed to BET bromodomain inhibition by JQ1 became sensitive to combination therapies targeting RTKs, PI3K or ERK signaling [100].

Other multi-targeted approaches

Finally, combination strategies targeting other signaling pathways, e.g. the angiogenesis pathway and intrinsic (mitochondrial) apoptotic pathway as well as combinations with immunotherapy should be investigated.

During the last two decades, anti-angiogenic therapy has proven to be an effective strategy in ovarian cancer. Bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor, has been added to standard platinum- and taxane-based chemotherapy regimens for a select subgroup of patients [2]. Moreover, IGF-1R signaling pathway has been implicated in bevacizumab resistance.

Dual VEGF and IGF-1R inhibition by bevacizumab and cixutumumab enhanced tumor growth inhibition in ovarian cancer cells and was more effective than either treatment alone [102]. These results suggest combining VEGF inhibitors with IGF-1R/IR inhibitors is a possible treatment strategy worth to investigate.

In addition to VEGF inhibition, combination strategies with inhibitors of the intrinsic apoptotic pathway should be further investigated. IGF-1R and IR signaling protects cells from apoptosis via the intrinsic (mitochondrial) apoptotic pathway by PI3K-AKT and ERK pathway activation [103]. Signaling via these two pathways inhibits pro-apoptotic Bcl-2 family member BAD by maintaining its phosphorylation status [103]. Phosphorylation of BAD prevents its heterodimerization with the anti-apoptotic Bcl-2 family members Bcl-x_L and Bcl-2. This enables Bcl-xL and Bcl-2 to exert their anti-apoptotic function by maintaining the mitochondrial integrity [104]. In addition, signaling via PI3K-AKT or ERK promotes transcription of anti-apoptotic Bcl-2 family member Mcl-1 whereas it inhibits transcription of pro-apoptotic Bcl-2 family members including Bim [105]. Anti-apoptotic Bcl-2 family members Bcl-x_L and Mcl-1 and pro-apoptotic Bcl-2 family member Bim are considered important targets in ovarian cancer [105]. Recently, it was shown that combined PI3K-AKT and ERK pathway inhibition sensitized ovarian cancer cells to Bcl-x_L inhibition [105]. These results indicate that a combination of PI3K-AKT and ERK pathway inhibition, for instance by targeting IGF-1R/IR, with inhibitors of the intrinsic apoptotic pathway may be a valid novel therapeutic strategy for ovarian cancer.

Currently, immune checkpoint inhibitors are under intense investigation in the clinic for many types of cancer, including ovarian cancer, and results are promising [106]. It has become clear that in addition to the intended effect on downstream signaling pathways, many targeted treatment strategies also directly modulate immune responses. For example, PI3K-AKT pathway inhibitors have been shown to sensitize cells to immunotherapy[107]. Therefore, combining IGF-1R/IR inhibitors with immunotherapy may be worth investigating.

Conclusions and perspectives

Ovarian cancer remains the most lethal gynecological cancer, therefore novel targeted strategies are warranted. The IGF-1R/IR signaling pathway plays an important role in development, maintenance, progression, survival and chemotherapeutic response of ovarian cancer and there is ample pre-clinical evidence demonstrating the therapeutic relevance of IGF-1R/IR targeted strategies in ovarian cancer. Therefore, IGF-1R/IR targeting strategies entered the clinic with high expectations. However, in the clinic these high expectations were tempered by lack of efficacy. The question now remains whether targeting the IGF-1R/IR signaling pathway still has any therapeutic potential.

In retrospect, single IGF-1R targeting strategies were not sufficient as IGF-1R signaling loss is compensated by IR(-A) as well as multiple other RTKs e.g. EGFR, HER2, HER3 and c-Met. Dual IGF-1R/IR RTK inhibitors tackle the problem of compensation by IR but are equally compensated by other RTKs e.g. HER2 and lack clinical efficacy as well. These RTK compensatory mechanisms are bidirectional, therefore multi-targeted approaches, either aimed at preventing this RTK system rewiring e.g. BET bromodomain inhibitors or multi-targeted approaches aimed at targeting multiple RTKs in combination with downstream pathway inhibitors of PI3K, AKT and ERK pathways may have more therapeutic potential.

In the clinic dual IGF-1R/IR inhibitors are well tolerated demonstrating IR targeting is feasible. IR is highly expressed in ovarian cancer tissues with IR-A being the predominant isoform. The mechanisms involved in this preferential IR-A expression in cancer cells are largely unknown and warrant further investigation. Further, the importance of IR-B in cancer development and progression cannot be excluded. As single IGF-1R and dual IGF-1R/IR targeting strategies failed in the clinic, single IR targeting may be worth investigating. As such the development of specific IR targeting strategies deserves further exploration.

Recently, even more complexity was added to the IGF-1R signaling pathway. IGF-1R and possibly IR are proposed to function as RTK/GPCR hybrid receptors. In line with this, both IGF-1R and IR are now considered to be dependence receptors. These observations advocate the use of IGF-capturing antibodies in the clinic.

Finally, targeting a single node within the PI3K and ERK signaling pathway is limited by compensatory mechanisms within and between these pathways. Therefore, combination strategies with immunotherapy or inhibitors of other signaling pathways, e.g. angiogenesis, intrinsic apoptosis and GPCR signaling, should be investigated. For successful targeting of the IGF-1R/IR signaling pathway in the clinic, multiple-targeted approaches should be explored in pre-selected patient cohorts, which warrant further research to identify biomarkers of treatment efficacy.

Search strategy

Literature was searched (until August 2017), with the following search criteria in PubMed: [insulin-like growth factor receptor or IGF-1R] combined with [insulin-like growth factor or IGF- system], [insulin receptor or IR], [insulin receptor isoforms or IR isoforms], [IR-A], [IR-B], [cancer], [IGF-I], [IGF-II], [insulin], [binding affinity] [ovarian cancer], [receptor heterodimerization], [downstream signaling], [targeting], [receptor hybrids], [receptor crosstalk] and variations thereof. Only studies published in the English language were included. Clinicaltrials.gov was used to evaluate completed and ongoing clinical trials with IGF-1R and IR targeted agents in ovarian cancer patients.

Funding

This work was supported by the Dutch Cancer Society, KWF grants (RUG 2011-5231 and RUG 2012-5477).

Conflict of interest

The authors declared that there is no conflict of interest.

References

[1] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9–29.
[2] Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol 2015;16:928–36.
[3] Ricci F, Broggini M, Damia G. Revisiting ovarian cancer preclinical models: implications for a better management of the disease. Cancer Treat Rev 2013;39:561–8.
[4] Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C, et al. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995–2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. Lancet 2011;377:127–38.
[5] Bookman MA, Brady MF, McGuire WP, Harper PG, Alberts DS, Friedlander M, et al. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup. J Clin Oncol 2009;27:1419–25.
[6] Chung C, Lee R. An update on current and emerging therapies for epithelial ovarian cancer: focus on poly(adenosine diphosphate-ribose) polymerase inhibition and antiangiogenesis. J Oncol Pharm Pract 2016;23(6):454–69.
[7] Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia. Nat Rev Cancer 2004;4:505–18.
[8] Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev 1999;20:761–87.

[9] Rother KI, Accili D. Role of insulin receptors and IGF receptors in growth and development. Pediatr Nephrol 2000;14:558–61.
[10] Giudice LC. Maternal-fetal conflict–lessons from a transgene. J Clin Invest 2002;110:307–9.
[11] Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915–28.
[12] Girnita L, Worrall C, Takahashi S, Seregard S, Girnita A. Something old, something new and something borrowed: emerging paradigm of insulin-like growth factor type 1 receptor (IGF-1R) signaling regulation. Cell Mol Life Sci 2014;71:2403–27.
[13] Crudden C, Ilic M, Suleymanova N, Worrall C, Girnita A, Girnita L. The dichotomy of the Insulin-like growth factor 1 receptor: RTK and GPCR: friend or foe for cancer treatment? Growth Horm IGF Res 2015;25:2–12.
[14] Baxter RC. IGF binding proteins in cancer: mechanistic and clinical insights. Nat Rev Cancer 2014;14:329–41.
[15] Yee D, Morales FR, Hamilton TC, Von Hoff DD. Expression of insulin-like growth factor I, its binding proteins, and its receptor in ovarian cancer. Can Res 1991;51:5107–12.
[16] Resnicoff M, Ambrose D, Coppola D, Rubin R. Insulin-like growth factor-1 and its receptor mediate the autocrine proliferation of human ovarian carcinoma cell lines. Lab Invest 1993;69:756–60.
[17] Ouban A, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. Hum Pathol 2003;34:803–8.
[18] Bruchim I, Werner H. Targeting IGF-1 signaling pathways in gynecologic malignancies. Expert Opin Ther Targets 2013;17:307–20.
[19] Baserga R. The decline and fall of the IGF-I receptor. J Cell Physiol 2013;228:675–9.
[20] Crudden C, Girnita A, Girnita L. Targeting the IGF-1R: the tale of the tortoise and the hare. Front Endocrinol (Lausanne) 2015;6:64.
[21] De Meyts P, Whittaker J. Structural biology of insulin and IGF1 receptors: implications for drug design. Nat Rev Drug Discov 2002;1:769–83.
[22] Kara I, Poggi M, Bonardo B, Govers R, Landrier JF, Tian S, et al. The paired basic amino acid-cleaving enzyme 4 (PACE4) is involved in the maturation of insulin receptor isoform B: an opportunity to reduce the specific insulin receptor-dependent effects of insulin-like growth factor 2 (IGF2). J Biol Chem 2015;290:2812–21.
[23] Kovacina KS, Roth RA. Characterization of the endogenous insulin receptor-related receptor in neuroblastomas. J Biol Chem 1995;270:1881–7.
[24] Jui HY, Accili D, Taylor SI. Characterization of a hybrid receptor formed by dimerization of the insulin receptor-related receptor (IRR) with the insulin receptor (IR): coexpression of cDNAs encoding human IRR and human IR in NIH-3T3 cells. Biochemistry 1996;35:14326–30.
[25] Bailyes EM, Nave BT, Soos MA, Orr SR, Hayward AC, Siddle K. Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem J 1997;327(Pt 1):209–15.
[26] Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, Belfiore A. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 2002;277:39684–95.
[27] Benyoucef S, Surinya KH, Hadaschik D, Siddle K. Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and the alternatively spliced exon 11 sequence to ligand binding and receptor activation. Biochem J 2007;403:603–13.
[28] Blanquart C, Achi J, Issad T. Characterization of IRA/IRB hybrid insulin receptors using bioluminescence resonance energy transfer. Biochem Pharmacol 2008;76:873–83.
[29] Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, Costantino A, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 1999;19:3278–88.
[30] Buck E, Gokhale PC, Koujak S, Brown E, Eyzaguirre A, Tao N, et al. Compensatory insulin receptor (IR) activation on inhibition of insulin-like growth factor-1 receptor (IGF-1R): rationale for cotargeting IGF-1R and IR in cancer. Mol Cancer Ther 2010;9:2652–64.
[31] Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr Rev 2009;30:586–623.
[32] Deyev IE, Popova NV, Petrenko AG. Determination of alkali-sensing parts of the insulin receptor-related receptor using the bioinformatic approach. Acta Naturae 2015;7:80–6.
[33] El-Shewy HM, Luttrell LM. Insulin-like growth factor-2/mannose-6 phosphate receptors. Vitam Horm 2009;80:667–97.
[34] Slaaby R, Schaffer L, Lautrup-Larsen I, Andersen AS, Shaw AC, Mathiasen IS, et al. Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J Biol Chem 2006;281:25869–74.
[35] Whittaker L, Hao C, Fu W, Whittaker J. High-affinity insulin binding: insulin interacts with two receptor ligand binding sites. Biochemistry 2008;47:12900–9.
[36] Leibiger B, Leibiger IB, Moede T, Kemper S, Kulkarni RN, Kahn CR, et al. Selective insulin signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic beta cells. Mol Cell 2001;7:559–70.
[37] Pandini G, Medico E, Conte E, Sciacca L, Vigneri R, Belfiore A. Differential gene expression induced by insulin and insulin-like growth factor-II through the insulin receptor isoform A. J Biol Chem 2003;278:42178–89.
[38] Denley A, Carroll JM, Brierley GV, Cosgrove L, Wallace J, Forbes B, et al. Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors. Mol Cell Biol 2007;27:3569–77.
[39] Sacco A, Morcavallo A, Pandini G, Vigneri R, Belfiore A. Differential signaling activation by insulin and insulin-like growth factors I and II upon binding to insulin receptor isoform A. Endocrinology 2009;150:3594–602.
[40] Morcavallo A, Gaspari M, Pandini G, Palummo A, Cuda G, Larsen MR, et al. Research resource: new and diverse substrates for the insulin receptor isoform A revealed by quantitative proteomics after stimulation with IGF-II or insulin. Mol Endocrinol 2011;25:1456–68.
[41] Vogt B, Carrascosa JM, Ermel B, Ullrich A, Haring HU. The two isotypes of the human insulin receptor (HIR-A and HIR-B) follow different internalization kinetics. Biochem Biophys Res Commun 1991;177:1013–8.
[42] Morcavallo A, Genua M, Palummo A, Kletvikova E, Jiracek J, Brzozowski AM, et al. Insulin and insulin-like growth factor II differentially regulate endocytic sorting and stability of insulin receptor isoform A. J Biol Chem 2012;287:11422–36.
[43] Morcavallo A, Stefanello M, Iozzo RV, Belfiore A, Morrione A. Ligand-mediated endocytosis and trafficking of the insulin-like growth factor receptor I and insulin receptor modulate receptor function. Front Endocrinol (Lausanne) 2014;5:220.
[44] Uhles S, Moede T, Leibiger B, Berggren PO, Leibiger IB. Isoform-specific insulin receptor signaling involves different plasma membrane domains. J Cell Biol 2003;163:1327–37.
[45] Beck EP, Russo P, Gliozzo B, Jaeger W, Papa V, Wildt L, et al. Identification of insulin and insulin-like growth factor I (IGF I) receptors in ovarian cancer tissue. Gynecol Oncol 1994;53:196–201.
[46] Kalli KR, Falowo OI, Bale LK, Zschunke MA, Roche PC, Conover CA. Functional insulin receptors on human epithelial ovarian carcinoma cells: implications for IGF-II mitogenic signaling. Endocrinology 2002;143:3259–67.
[47] Huang Z, Wen Y, Shandilya R, Marks JR, Berchuck A, Murphy SK. High throughput detection of M6P/IGF2R intronic hypermethylation and LOH in ovarian cancer. Nucleic Acids Res 2006;34:555–63.
[48] Pejovic T, Pande NT, Mori M, Mhawech-Fauceglia P, Harrington C, Mongoue-Tchokote S, et al. Expression profiling of the ovarian surface kinome reveals candidate genes for early neoplastic changes. Transl Oncol 2009;2:341–9.
[49] Eckstein N, Servan K, Hildebrandt B, Politz A, von Jonquieres G, Wolf-Kummeth S, et al. Hyperactivation of the insulin-like growth factor receptor I signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. Can Res 2009;69:2996–3003.
[50] Beltran PJ, Calzone FJ, Mitchell P, Chung YA, Cajulis E, Moody G, et al. Ganitumab (AMG 479) inhibits IGF-II-dependent ovarian cancer growth and potentiates platinum-based chemotherapy. Clin Cancer Res 2014;20:2947–58.
[51] Zhang B, Roth RA. The insulin receptor-related receptor. Tissue expression, ligand binding specificity, and signaling capabilities. J Biol Chem 1992;267:18320–8.
[52] Brokaw J, Katsaros D, Wiley A, Lu L, Su D, Sochirca O, et al. IGF-I in epithelial ovarian cancer and its role in disease progression. Growth Factors 2007;25:346–54.
[53] King ER, Zu Z, Tsang YT, Deavers MT, Malpica A, Mok SC, et al. The insulin-like growth factor 1 pathway is a potential therapeutic target for low-grade serous ovarian carcinoma. Gynecol Oncol 2011;123:13–8.
[54] Sayer RA, Lancaster JM, Pittman J, Gray J, Whitaker R, Marks JR, et al. High insulin-like growth factor-2 (IGF-2) gene expression is an independent predictor of poor survival for patients with advanced stage serous epithelial ovarian cancer. Gynecol Oncol 2005;96:355–61.
[55] Huang GS, Brouwer-Visser J, Ramirez MJ, Kim CH, Hebert TM, Lin J, et al. Insulin-like growth factor 2 expression modulates Taxol resistance and is a candidate biomarker for reduced disease-free survival in ovarian cancer. Clin Cancer Res 2010;16:2999–3010.
[56] Karasik A, Menczer J, Pariente C, Kanety H. Insulin-like growth factor-I (IGF-I) and IGF-binding protein-2 are increased in cyst fluids of epithelial ovarian cancer. J Clin Endocrinol Metab 1994;78:271–6.
[57] Gotlieb WH, Bruchim I, Gu J, Shi Y, Camirand A, Blouin MJ, et al. Insulin-like growth factor receptor I targeting in epithelial ovarian cancer. Gynecol Oncol 2006;100:389–96.
[58] Koti M, Gooding RJ, Nuin P, Haslehurst A, Crane C, Weberpals J, et al. Identification of the IGF1/PI3K/NF kappaB/ERK gene signalling networks associated with chemotherapy resistance and treatment response in high-grade serous epithelial ovarian cancer. BMC Cancer 2013;13:549. 2407-13-549.
[59] Mosig RA, Lobl M, Senturk E, Shah H, Cohen S, Chudin E, et al. IGFBP-4 tumor and serum levels are increased across all stages of epithelial ovarian cancer. J Ovarian Res 2012;5:3. 2215-5-3.
[60] Wang H, Rosen DG, Wang H, Fuller GN, Zhang W, Liu J. Insulin-like growth factor-binding protein 2 and 5 are differentially regulated in ovarian cancer of different histologic types. Mod Pathol 2006;19:1149–56.
[61] Huang YF, Cheng WF, Wu YP, Cheng YM, Hsu KF, Chou CY. Circulating IGF system and treatment outcome in epithelial ovarian cancer. Endocr Relat Cancer 2014;21:217–29.
[62] Hwang JR, Cho YJ, Lee Y, Park Y, Han HD, Ahn HJ, et al. The C-terminus of IGFBP-5 suppresses tumor growth by inhibiting angiogenesis. Sci Rep 2016;6:39334.

[63] Rho SB, Dong SM, Kang S, Seo SS, Yoo CW, Lee DO, et al. Insulin-like growth factor-binding protein-5 (IGFBP-5) acts as a tumor suppressor by inhibiting angiogenesis. Carcinogenesis 2008;29:2106–11.
[64] Muller M, Dietel M, Turzynski A, Wiechen K. Antisense phosphorothioate oligodeoxynucleotide down-regulation of the insulin-like growth factor I receptor in ovarian cancer cells. Int J Cancer 1998;77:567–71.
[65] Wang Y, Hailey J, Williams D, Wang Y, Lipari P, Malkowski M, et al. Inhibition of insulin-like growth factor-I receptor (IGF-IR) signaling and tumor cell growth by a fully human neutralizing anti-IGF-IR antibody. Mol Cancer Ther 2005;4:1214–21.
[66] An Y, Cai Y, Guan Y, Cai L, Yang Y, Feng X, et al. Inhibitory effect of small interfering RNA targeting insulin-like growth factor-I receptor in ovarian cancer OVCAR3 cells. Cancer Biother Radiopharm 2010;25:545–52.
[67] Tang J, Li J, Zeng G, Tang Y, Tian W, He J, et al. Antisense oligonucleotide suppression of human IGF-1R inhibits the growth and survival of in vitro cultured epithelial ovarian cancer cells. J Ovarian Res 2013;6:71. 2215-6-71.
[68] Carboni JM, Wittman M, Yang Z, Lee F, Greer A, Hurlburt W, et al. BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR. Mol Cancer Ther 2009;8:3341–9.
[69] Ray-Coquard I, Haluska P, O'Reilly S, Cottu PH, Elit L, Provencher DM, et al. A multicenter open-label phase II study of the efficacy and safety of ganitumab (AMG 479), a fully human monoclonal antibody against insulin-like growth factor type 1 receptor (IGF-1R) as second-line therapy in patients with recurrent platinum-sensitive ovarian cancer. JCO 2013;31:5515.
[70] Brana I, Berger R, Golan T, Haluska P, Edenfield J, Fiorica J, et al. A parallel-arm phase I trial of the humanised anti-IGF-1R antibody dalotuzumab in combination with the AKT inhibitor MK-2206, the mTOR inhibitor ridaforolimus, or the NOTCH inhibitor MK-0752, in patients with advanced solid tumours. Br J Cancer 2014;111:1932–44.
[71] Harb WA, Sessa C, Hirte HW, Kaye SB, Banerjee SN, Christinat A, et al. Final results of a phase I study evaluating the combination of linsitinib, a dual inhibitor of insulin-like growth factor-1 receptor (IGF-1R), and insulin receptor (IR) with weekly paclitaxel (PAC) in patients (Pts) with advanced solid tumors. JCO 2013;31:e13502.
[72] Gilmore AP, Valentijn AJ, Wang P, Ranger AM, Bundred N, O'Hare MJ, et al. Activation of BAD by therapeutic inhibition of epidermal growth factor receptor and transactivation by insulin-like growth factor receptor. J Biol Chem 2002;277:27643–50.
[73] Ahmad T, Farnie G, Bundred NJ, Anderson NG. The mitogenic action of insulin-like growth factor I in normal human mammary epithelial cells requires the epidermal growth factor receptor tyrosine kinase. J Biol Chem 2004;279:1713–9.
[74] Morgillo F, Woo JK, Kim ES, Hong WK, Lee HY. Heterodimerization of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib. Can Res 2006;66:10100–11.
[75] Riedemann J, Takiguchi M, Sohail M, Macaulay VM. The EGF receptor interacts with the type 1 IGF receptor and regulates its stability. Biochem Biophys Res Commun 2007;355:707–14.
[76] van der Veeken J, Oliveira S, Schiffelers RM, Storm G, van Bergen En Henegouwen PM, Roovers RC. Crosstalk between epidermal growth factor receptor- and insulin-like growth factor-1 receptor signaling: implications for cancer therapy. Curr Cancer Drug Targets 2009;9:748–60.
[77] Balana ME, Labriola L, Salatino M, Movsichoff F, Peters G, Charreau EH, et al. Activation of ErbB-2 via a hierarchical interaction between ErbB-2 and type I insulin-like growth factor receptor in mammary tumor cells. Oncogene 2001;20:34–47.
[78] Huang X, Gao L, Wang S, McManaman JL, Thor AD, Yang X, et al. Heterotrimerization of the growth factor receptors erbB2, erbB3, and insulin-like growth factor-i receptor in breast cancer cells resistant to herceptin. Can Res 2010;70:1204–14.
[79] Varkaris A, Gaur S, Parikh NU, Song JH, Dayyani F, Jin JK, et al. Ligand-independent activation of MET through IGF-1/IGF-1R signaling. Int J Cancer 2013;133:1536–46.
[80] Fafalios A, Ma J, Tan X, Stoops J, Luo J, Defrances MC, et al. A hepatocyte growth factor receptor (Met)-insulin receptor hybrid governs hepatic glucose metabolism. Nat Med 2011;17:1577–84.
[81] Jones HE, Gee JM, Barrow D, Tonge D, Holloway B, Nicholson RI. Inhibition of insulin receptor isoform-A signalling restores sensitivity to gefitinib in previously de novo resistant colon cancer cells. Br J Cancer 2006;95:172–80.
[82] Chettouh H, Fartoux L, Aoudjehane L, Wendum D, Claperon A, Chretien Y, et al. Mitogenic insulin receptor-A is overexpressed in human hepatocellular carcinoma due to EGFR-mediated dysregulation of RNA splicing factors. Can Res 2013;73:3974–86.
[83] Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. Can Res 2005;65:11118–28.
[84] Haluska P, Carboni JM, TenEyck C, Attar RM, Hou X, Yu C, et al. HER receptor signaling confers resistance to the insulin-like growth factor-I receptor inhibitor, BMS-536924. Mol Cancer Ther 2008;7:2589–98.
[85] Jia Y, Zhang Y, Qiao C, Liu G, Zhao Q, Zhou T, et al. IGF-1R and ErbB3/HER3 contribute to enhanced proliferation and carcinogenesis in trastuzumab-resistant ovarian cancer model. Biochem Biophys Res Commun 2013;436:740–5.
[86] Dalle S, Ricketts W, Imamura T, Vollenweider P, Olefsky JM. Insulin and insulin-like growth factor I receptors utilize different G protein signaling components. J Biol Chem 2001;276:15688–95.
[87] Fassnacht M, Berruti A, Baudin E, Demeure MJ, Gilbert J, Haak H, et al. Linsitinib (OSI-906) versus placebo for patients with locally advanced or metastatic adrenocortical carcinoma: a double-blind, randomised, phase 3 study. Lancet Oncol 2015;16:426–35.
[88] Louvi A, Accili D, Efstratiadis A. Growth-promoting interaction of IGF-II with the insulin receptor during mouse embryonic development. Dev Biol 1997;189:33–48.
[89] Morrione A, Valentinis B, Xu SQ, Yumet G, Louvi A, Efstratiadis A, et al. Insulin-like growth factor II stimulates cell proliferation through the insulin receptor. Proc Natl Acad Sci USA 1997;94:3777–82.
[90] Huang J, Morehouse C, Streicher K, Higgs BW, Gao J, Czapiga M, et al. Altered expression of insulin receptor isoforms in breast cancer. PLoS ONE 2011;6:e26177.
[91] Garofalo C, Manara MC, Nicoletti G, Marino MT, Lollini PL, Astolfi A, et al. Efficacy of and resistance to anti-IGF-1R therapies in Ewing's sarcoma is dependent on insulin receptor signaling. Oncogene 2011;30:2730–40.
[92] Talukdar I, Sen S, Urbano R, Thompson J, Yates 3rd JR, Webster NJ. hnRNP A1 and hnRNP F modulate the alternative splicing of exon 11 of the insulin receptor gene. PLoS ONE 2011;6:e27869.
[93] Boucher J, Macotela Y, Bezy O, Mori MA, Kriauciunas K, Kahn CR. A kinase-independent role for unoccupied insulin and IGF-1 receptors in the control of apoptosis. Sci Signal 2010;3:ra87.
[94] Gao J, Chesebrough JW, Cartlidge SA, Ricketts SA, Incognito L, Veldman-Jones M, et al. Dual IGF-I/II-neutralizing antibody MEDI-573 potently inhibits IGF signaling and tumor growth. Can Res 2011;71:1029–40.
[95] Friedbichler K, Hofmann MH, Kroez M, Ostermann E, Lamche HR, Koessl C, et al. Pharmacodynamic and antineoplastic activity of BI 836845, a fully human IGF ligand-neutralizing antibody, and mechanistic rationale for combination with rapamycin. Mol Cancer Ther 2014;13:399–409.
[96] Iguchi H, Nishina T, Nogami N, Kozuki T, Yamagiwa Y, Yagawa K. Phase I dose-escalation study evaluating safety, tolerability and pharmacokinetics of MEDI-573, a dual IGF-I/II neutralizing antibody, in Japanese patients with advanced solid tumours. Invest New Drugs 2015;33:194–200.
[97] Fitzgerald JB, Johnson BW, Baum J, Adams S, Iadevaia S, Tang J, et al. MM-141, an IGF-IR- and ErbB3-directed bispecific antibody, overcomes network adaptations that limit activity of IGF-IR inhibitors. Mol Cancer Ther 2014;13:410–25.
[98] Stratikopoulos EE, Dendy M, Szabolcs M, Khaykin AJ, Lefebvre C, Zhou MM, et al. Kinase and BET inhibitors together clamp inhibition of PI3K signaling and overcome resistance to therapy. Cancer Cell 2015;27:837–51.
[99] Stuhlmiller TJ, Miller SM, Zawistowski JS, Nakamura K, Beltran AS, Duncan JS, et al. Inhibition of lapatinib-induced kinome reprogramming in ERBB2-positive breast cancer by targeting BET family bromodomains. Cell Rep 2015;11:390–404.
[100] Kurimchak AM, Shelton C, Duncan KE, Johnson KJ, Brown J, O'Brien S, et al. Resistance to BET bromodomain inhibitors is mediated by kinome reprogramming in ovarian cancer. Cell Rep 2016;16:1273–86.
[101] Baratta MG, Schinzel AC, Zwang Y, Bandopadhayay P, Bowman-Colin C, Kutt J, et al. An in-tumor genetic screen reveals that the BET bromodomain protein, BRD4, is a potential therapeutic target in ovarian carcinoma. Proc Natl Acad Sci USA 2015;112:232–7.
[102] Shao M, Hollar S, Chambliss D, Schmitt J, Emerson R, Chelladurai B, et al. Targeting the insulin growth factor and the vascular endothelial growth factor pathways in ovarian cancer. Mol Cancer Ther 2012;11:1576–86.
[103] Peruzzi F, Prisco M, Dews M, Salomoni P, Grassilli E, Romano G, et al. Multiple signaling pathways of the insulin-like growth factor 1 receptor in protection from apoptosis. Mol Cell Biol 1999;19:7203–15.
[104] Deng J. How to unleash mitochondrial apoptotic blockades to kill cancers? Acta Pharm Sin B 2017;7:18–26.
[105] Petigny-Lechartier C, Duboc C, Jebahi A, Louis MH, Abeilard E, Denoyelle C, et al. The mTORC1/2 Inhibitor AZD8055 strengthens the efficiency of the MEK inhibitor trametinib to reduce the Mcl-1/[Bim and Puma] ratio and to sensitize ovarian carcinoma cells to ABT-737. Mol Cancer Ther 2017;16:102–15.
[106] Ventriglia J, Paciolla I, Pisano C, Cecere SC, Di Napoli M, Tambaro R, et al. Immunotherapy in ovarian, endometrial and cervical cancer: state of the art and future perspectives. Cancer Treat Rev 2017;59:109–16.
[107] Vanneman M, Dranoff G. Combining immunotherapy and targeted therapies in cancer treatment. Nat Rev Cancer 2012;12:237–51.
[108] Yamaguchi Y, Flier JS, Benecke H, Ransil BJ, Moller DE. Ligand-binding properties of the two isoforms of the human insulin receptor. Endocrinology 1993;132:1132–8.
[109] Denley A, Bonython ER, Booker GW, Cosgrove LJ, Forbes BE, Ward CW, et al. Structural determinants for high-affinity binding of insulin-like growth factor II to insulin receptor (IR)-A, the exon 11 minus isoform of the IR. Mol Endocrinol 2004;18:2502–12.
[110] Hughes J, Frago S, Buhnemann C, Carter EJ, Hassan AB. Maternal transmission of a humanised Igf2r allele results in an Igf2 dependent hypomorphic and non-viable growth phenotype. PLoS ONE 2013;8:e57270.